植 物 分 类 与 资 源 学 报 2015, **37** (1): 46~54
Plant Diversity and Resources DOI: 10.7677/ynzwyj201514037

高山植物圆锥南芥的光合系统耐热性及其修复机制*

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摘要: 高温胁迫包括极端高温和中高温, 严重影响了植物的一系列生理活动, 尤其是光合作用, 而植物应对极端高温和中高温胁迫具有不同的策略。高山植物因长期生长于相对寒冷的环境中, 相比而言应缺少对高温胁迫的适应机制。本文以圆锥南芥作为一种高山模式植物来探索其在中高温下是否表现出耐热能力, 如果具有耐热能力, 那么在光合方面与拟南芥存在怎样的差异。研究发现, 圆锥南芥在中高温处理后具有更高的光化学效率及快速可逆的恢复过程, 表现出了较强的耐热能力。两物种的 F_0 没有明显的差异, 而圆锥南芥在热处理后及恢复过程中具有更高的 F_m, 促进其快速光合修复。在热处理后, 非光化学能量耗散快速瞬时上升, 及时保护光系统 II 免受光损伤和热伤害, 另外, HSP101 蛋白迅速诱导可能启动了光化学修复。最后, 圆锥南芥在严重高温处理后具有更高的存活率再次验证了它在中高温下的耐热能力。结果表明, 圆锥南芥具有更耐热的光合系统以及有效的光合修复机制来耐受中高温胁迫。

关键词: 高温胁迫; 中高温胁迫; 光合作用; 圆锥南芥; 非光化学能量耗散; 热激蛋白
中图分类号: Q 945 **文献标志码**: A **文章编号**: 2095-0845(2015)01-046-09

The Thermotolerance and Repair Mechanism of Photosystem in Alpine Plant *Arabis paniculata* (Cruciferae)*

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Abstract: The heat stress associated with extremely and moderately high temperatures affects a series of physiological activities in plants especially photosynthesis. However, it is proposed that the plants use different photosynthetic strategy to deal with extreme and moderate heat stresses. Most reports focus on the cold tolerant ability but thermotolerance of alpine plants. In the present study, we used the alpine plant *Arabis paniculata* as a model alpine plant to examine whether its capacity for heat tolerance is exhibited under moderate heat stress and, if so, how this capacity is related to differences in its photosynthesis compared with that of its close relative *Arabidopsis thaliana*. We found that *A. paniculata* had high photochemical efficiency at a moderately high temperature and a rapid reversible recovery process, which reflected substantial heat tolerance. Despite no obvious difference in F_0 between the two species, the higher F_m values after heat treatment and recovery in *A. paniculata* than in *A. thaliana* facilitated the rapid photochemical recovery. A rapid and transient increase in non-photochemical quenching after moderate heat stress provided timely protection for PSII against the damage caused by heat and light. The rapid accumulation of heat shock protein 101 upon exposure to moderately high temperatures might initiate photochemical repair. Finally, the high rate of survival of *A. paniculata* after severe heat treatment attested to the substantial heat tolerance of its photosynthetic machinery under moderate stress. Our results indicated that a highly heat-tolerant photosystem and effective photochemi-

* Funding: NSFC (31300251) and XiBuZhiGuang Project
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cal repair mechanism contribute to the capacity of *A. paniculata* to tolerate moderate heat stress.

Key words: Heat stress; Moderate heat stress; Photosynthesis; *Arabis paniculata*; Non-photochemical quenching; Heat shock protein

Heat stress caused by the exposure of plants to temperatures beyond their optimum is a major factor that limits crop production worldwide (Hall, 2010). High temperature can affect many physiological activities throughout the life cycles of plants. Photosynthesis is one of the most heat-sensitive process in plants and is often completely inhibited before the symptoms of other injuries appear. Heat stress can be classified into moderate and extreme according to duration of stress, time of the day at which it occurs and co-exposure to other stresses, each of which involves different coping mechanisms and adaptation strategies (Berry and Bjorkman, 1980; Blum, 1988). Such temperature stress occurs regularly in alpine environments (Körner, 2003). An extremely high temperature could cause severe cellular injuries and even a catastrophic collapse of cellular organisation within minutes (Schöffl *et al.*, 1999). However, significant and rapid reversible changes in photosynthetic metabolism caused by moderately high leaf temperature might differ from irreversible damage brought about by severe heat stress. These changes under moderate heat stress can help us understand how heat is tolerated by photosynthetic systems and provide insight into how plants could be made more heat-tolerant through genetic modification (Sharkey and Zhang, 2010).

Photosystem II (PSII), as a protein complex involved in photosynthesis is shown to be partially inhibited by a moderately high temperature (Song *et al.*, 2010). Chlorophyll fluorescence is a sensitive and reliable indicator of the changes caused by heat stress in components of the photosynthetic apparatus, such as PSII (Krause and Weis, 1991; Govindje, 1995; Strasser, 1997). The ratio of variable fluorescence to maximum fluorescence (F_v/F_m), the minimal fluorescence (F_0), and the maximal fluorescence (F_m) are important physiological parameters that are closely related to thermotolerance (Yamada *et al.*, 1996). For example, in bean plants, the initial F_0 level clearly increases after heat treatment and then declines 4 h after recovery (Petkova *et al.*, 2007). In oak leaves, PSII could be protected from heat-dependent photo-inhibition via NPQ, which dissipates excess excitation energy (Haldimann and Feller, 2004). Allakhverdiev *et al.* (2008) also found that exposure to a moderately high temperature did not result in severe damage to PSII, but it did inhibit its repair. Most reported studies concentrated on the process immediately after heat shock, but there is little information about the PSII changes during the post-heat stress recovery process. However, the recovery from heat stress is very significant for plants to survive in complex and variable environments. Thermal inactivation of PSII is slowly reversible and the recovery process is known to take several days (Seemann *et al.*, 1984; Bilger *et al.*, 1987; Karim *et al.*, 1999). It is vitally important to study the exact photosynthetic response over the period from heat treatment to the recovery process lasting several days.

The photochemical response to moderate heat stress and during the subsequent recovery process might be closely associated with certain biochemical activities. For instance, heat shock proteins (HSPs) may help in the degradation of proteins damaged by heat stress (Parsell and Lindquist, 1993). Chloroplast HSPs play a role in the prevention of stress-related damage rather than in the repair of such damage (Downs *et al.*, 1999). However, whether HSP101 functions in the process of photochemical repair has not been described.

Arabis paniculata, a relative of *Arabidopsis thaliana*, lives in alpine environments that experience moderately high temperatures (about 30 ℃) in some seasons (Zheng *et al.*, 2011). Against the above

background, the current study is intended to answer the following questions: Can *A. paniculata* tolerate exposure moderate increases in temperature? If so, how does its photosynthetic machinery respond? In addition, are these traits consistent with its thermotolerance under an extremely high temperature? As such, the specific aim of this study was to use chlorophyll fluorescence to compare the heat tolerance of PSII in *A. paniculata* with that of *A. thaliana* after moderate heat treatment and during the subsequent recovery stage lasting several days. We next measured a biochemical factor that might affect the photochemical repair process, namely, the accumulation of HSP proteins, in both species after heat shock and during recovery. Finally, we verified the heat tolerance of *A. paniculata* under a moderately high temperature comparing the survival rates of these two species under severe heat stress.

1 Methods and materials

1.1 Plant material

Arabis alpina, which is synonymous with *A. paniculata*, distributed widely in most mountain systems in European, East Africa, Central Asia and so on (Koch *et al.*, 2006). Seeds of *A. paniculata* were collected from Lijiang mountain (North latitude 26.86 and East longitude 100.25) with a altitude of more than 2 500 m in Yunnan Province. *A. thaliana* is mainly originated from Europe, Asia, and northwestern Africa (http://en.wikipedia.org/wiki/Arabidopsis thaliana). All known ecotypes of *Arabidopsis* are not tolerant to abiotic stresses (Bressan *et al.*, 2001). All studies involved seedlings (9 days after germination with 2 leaves) and mature plants (4 weeks grown in soil with a rosette of more than 6 leaves) of *A. paniculata* and *A. thaliana* (Columbia ecotype). The HSP101-depleted mutant *hot-1* (Salk 066374) of *Arabidopsis* (Columbia ecotype) was described previously (Zhang *et al.*, 2010).

1.2 Plant growth and heat treatments

Seeds of *A. paniculata* and *A. thaliana* were sterilised with ethanol (75%) for 2 min and sodium hypochlorite (5%) for 2 min, followed by three washes with sterile distilled water. After surface sterilisation, the seeds were cold-stratified for four days at 4 ℃ and sown on Murashige and Skoog (MS) medium containing 1% sucrose. The seeds were germinated and grown at 22 ℃ with a 12 h light/12 h dark photoperiod with a photosynthetic photon flux density of 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 9 days. Plates with seedlings were treated at 37 ℃ for 2 h (72 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and then left at 22 ℃ (4 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 1 h, before 1 h of heat treatment at 48 ℃ in the dark, and then left at 22 ℃ (4 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 3 days of recovery. For the soil-grown plants, 4-week-old seedlings were exposed to 45 ℃ for 2 h in the dark and then left at 22 ℃ (4 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 3 days of recovery. For the survival rate test, 9-day-old plated seedlings were treated at 45 ℃ for 3 h in the dark and then left at 22 ℃ (4 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for recovery.

1.3 Measurement of chlorophyll fluorescence

Chlorophyll fluorescence was measured using an IMAGING-PAM chlorophyll fluorometer and the Imaging Win software application (Walz, Effeltrich, Germany), as described previously (Woo *et al.*, 2008). A dark-light induction curve was applied to assess dark- and light-adapted parameters. Plants were given a saturating pulse (>1 800 μmol photons $\cdot m^{-2} \cdot s^{-1}$) and the levels of F_0, F_m, and F_v/F_m were determined after 20 min of dark adaptation. F_v/F_m was calculated as $(F_m - F_0)/F_m$. False-colour images of the F_v/F_m parameter are presented through the Imaging Win software (Woo *et al.*, 2008). After 40 sec of exposure in the dark and a subsequent 6 min of actinic illumination (111 μmol photons $\cdot m^{-2} \cdot s^{-1}$) with saturating flashes at intervals of 20 seconds, the actual quantum yield of PSII photochemistry [$Y(II)$], the yield of regulated energy dissipation [$Y(NPQ)$], the yield of non-regulated energy dissipation [$Y(NO)$], and an estimate of the fraction of open PS II centres (qL) were obtained after another 6 min of light adaptation before taking the final measurements.

1.4 Protein extraction and immunoblotting of HSPs

Total protein was isolated according to a previously described procedure (Fan *et al.*, 1997). Seedlings were ground with homogenisation buffer (50 mmol · L^{-1} Tris-HCl, pH 7.5; 10 mmol · L^{-1} KCl, 1 mmol · L^{-1} ethylenediaminetetraacetic acid, 0.5 mmol · L^{-1} phenylmethylsulfonyl fluoride and 2 mmol · L^{-1} dithiothreitol) in a precooled mortar. After centrifugation at 7 000 r · min^{-1} for 10 min at 4 ℃, the amount of protein in the supernatant was determined at 595 nm using a dye-binding assay with Coomassie Brilliant Blue. The same amount of total protein was separated by sodium dodecyl sulfate polyacrylamide gel electropheresis analysis and then transferred onto polyvinylidene difluoride filters. These filters were first probed with HSP101-specific antibodies, and then incubated with a secondary antibody conjugated to alkaline phosphatase. HSPs were visualised by staining the blot for phosphatase activity. Each measurement was performed independently at least three times.

2 Results and discussion

2.1 *A. paniculata* exhibited superior photochemical efficiency after moderate heat treatment and more rapid recovery than *A. thaliana*

We detected the maximal photochemical efficiency in *A. paniculata* and *A. thaliana* under moderate heat shock and during the subsequent recovery period in order to compare the heat tolerance of PSII between these species. As shown in Fig. 1, F_v/F_m declined after all three heat treatments and increased reversibly after several days of recovery in both plants. This suggested that these three types of heat treatment constituted moderate stress for the plants. After 40 min at 48 ℃ with heat acclimation, F_v/F_m remained at about 0.4 in *A. paniculata*, but it declined to almost 0 in *A. thaliana* (Fig. 1A). With increasing time at high temperature, F_v/F_m declined to 0 after heat treatment but began to increase during the recovery stage in both plants. The speed of this increase was more rapid in *A. paniculata* than in *A. thaliana*. These results suggest the substantial thermotolerance of PSII during the early development of *A. paniculata* seedlings under moderate heat stress. To assess the thermotolerance of PSII of *A. paniculata* in mature plants we measured the F_v/F_m of 4-week-old soil-grown seedlings in *A. paniculata* and *A. thaliana* under a moderately high temperature (Fig. 1B). F_v/F_m declined slightly in *A. paniculata* and more substantially in *A. thaliana* after 2 h at 45 ℃, and it recovered to the normal state more rapidly in *A. paniculata* than in *A. thaliana*. All of these findings indicated the considerable heat tolerance of the photosynthetic machinery of *A. paniculata* at all development stages. This tolerance might help *A. paniculata* to adapt to the daily increases in temperature to the moderate levels that occur in alpine environments.

2.2 The heat response pattern of F_0 and F_m provided the basis for the rapid recovery of F_v/F_m in *A. paniculata*

In order to study the factors that affect the rapid reversible changes in F_v/F_m of *A. paniculata* under moderate heat stress, we measured F_0 and F_m in this species and in its relative *A. thaliana*. Both F_0 and F_m were measured after 1 h at 48 ℃ with heat acclimation and during the subsequent recovery stage (Fig. 2). After heat shock, the F_v/F_m ratio decreased to about 0.2 in *A. paniculata* and to 0 in *A. thaliana*. It then increased more rapidly in *A. paniculata* than in *A. thaliana* during the recovery stage. Meanwhile, a similar continuous increase in F_0 increased was observed in both plants throughout the periods of heat shock and recovery. Whereas F_m decreased after heat treatment, it tended to increase during the subsequent recovery stage in both plants. This less marked decline and greater increase of F_m might account for the more rapid recovery of F_v/F_m in *A. paniculata* than in *A. thaliana*. Whereas the increase in F_0 indicated reduced function of the light-harvesting complex, the decrease in F_m, which probably resulted from a change in the structure of PSII, suggested a decrease in its photochemical efficiency

(Mishra and Singhal, 1992; Petkova *et al.*, 2009). The strong thermotolerance of *A. paniculata* might be closely related to the thermostability of PSII under moderate heat treatment and the rapid reversible repair of the photosynthetic apparatus.

2.3 Non-photochemical quenching improved the recovery of actual photochemical efficiency by reducing photodamage in *A. paniculata*

Given that F_v/F_m might not comprehensively reflect actual photochemical activities, we next investigated

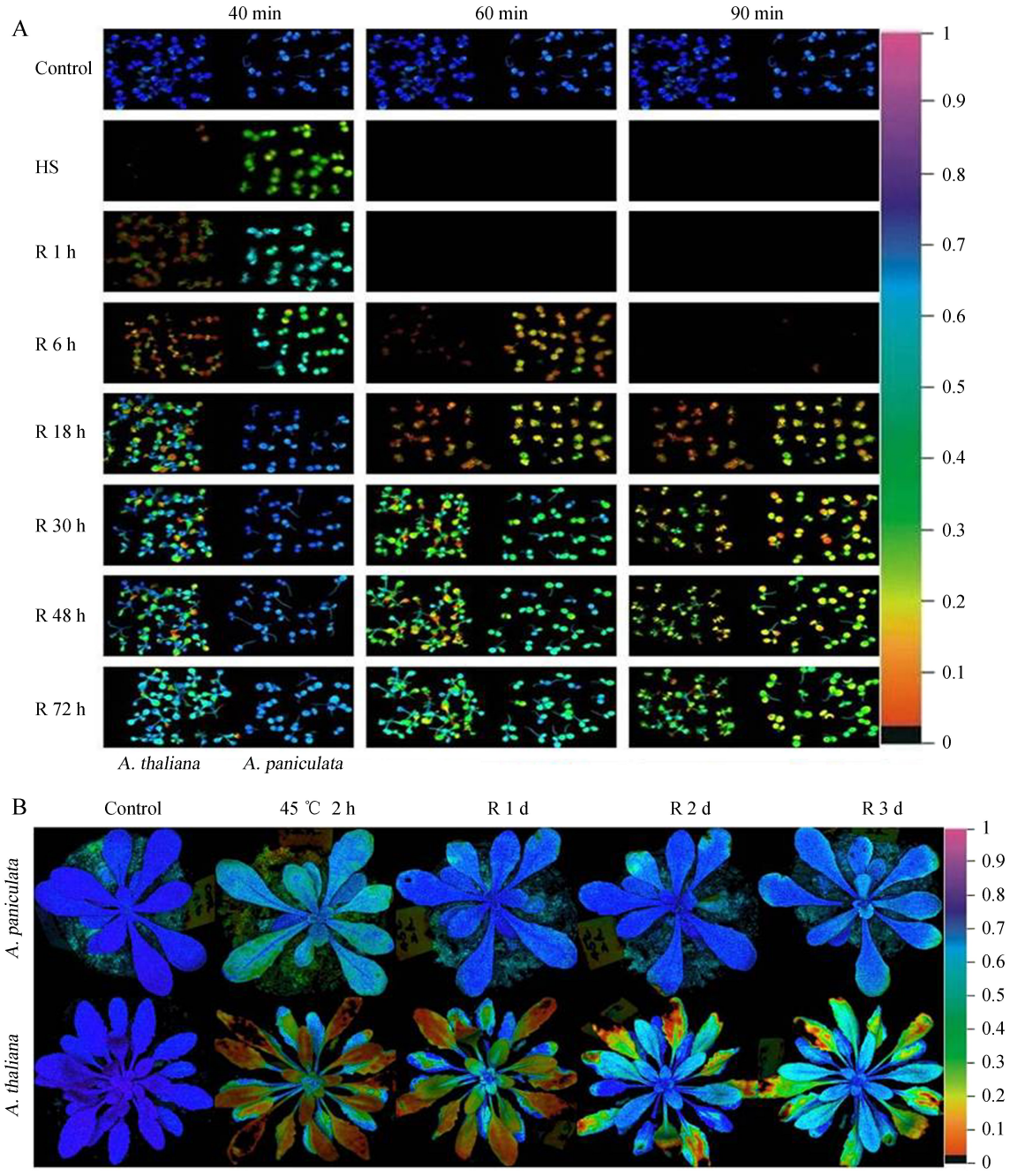

Fig. 1 A. False-colour imaging of the parameter that indicates maximum quantum yield of PSII (F_v/F_m) in seedlings of *A. paniculata* and *A. thaliana* after 1 h of exposure to 48 ℃ after heat acclimation (37 ℃ for 2 h, 72 μmol·m^{-2}·s^{-1}) and during the subsequent recovery period. "R 1 h" means 1 h of recovery at 22 ℃ after heat treatment, and so on; B. False-colour images of the maximum quantum yield of PSII (F_v/F_m) of 4-week-old soil-grown seedlings of *A. paniculata* and *A. thaliana* after 2 h of heat treatment at 45 ℃ and during the subsequent recovery period. "R 1 d" means 1 d of recovery at 22 ℃ after heat treatment, and so on

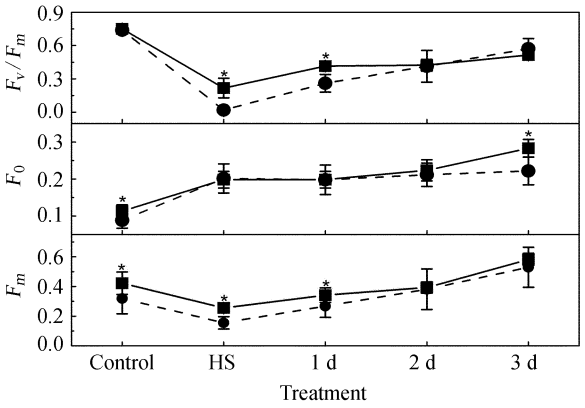

Fig. 2　The maximum quantum yield of PSII (F_v/F_m), the minimal fluorescence yield (F_0), and the maximal fluorescence yield (F_m) of plated seedlings of *A. paniculata* and *A. thaliana* after 1 h of heat treatment at 48 ℃ with heat acclimation (37 ℃ for 2 h, 72 μmol·m^{-2}·s^{-1}) and during the subsequent recovery period. Solid and dashed lines represent *A. paniculata* and *A. thaliana*, respectively. "1 d" means 1 d of recovery at 22 ℃ after heat treatment, and so on. An asterisk means that the value in *A. paniculata* is different from that in *A. thaliana* in the same treatment ($P < 0.05$)

the response pattern of actual photochemical efficiency $Y(II)$ after moderate heat treatment (Fig. 3). As shown in Fig. 3, the overall pattern of change of $Y(II)$ was consistent with that of F_v/F_m after heat treatment in both plants. Although the light energy absorbed by the plants could be used primarily for photochemical activities, some of it dissipated as heat by NPQ (Hendrickson *et al.*, 2004; Kramer *et al.*, 2004). After heat treatment, $Y(NPQ)$ increased to a peak in *A. paniculata*, but it declined to 0 in *A. thaliana*, and then returned to its respective control levels in both plants. This indicated that $Y(NPQ)$ protected the photosystem only transiently after heat treatment in order to improve the recovery of $Y(II)$ in *A. paniculata*. The pattern of change of *qL* was consistent with $Y(II)$ throughout this whole process in both plants. There was a more marked increase in $Y(NO)$ in *A. thaliana* than in *A. paniculata*. An increase in $Y(NO)$ to close to 1 in *A. thaliana* might indicate an almost complete breakdown in photochemistry. All of these results suggest that $Y(NPQ)$ might protect the photosynthetic apparatus by lessening the injuries caused by heat damage and photodamage in *A. paniculata*; the reduced level of damage might eventually accelerate the recovery of photochemical efficiency.

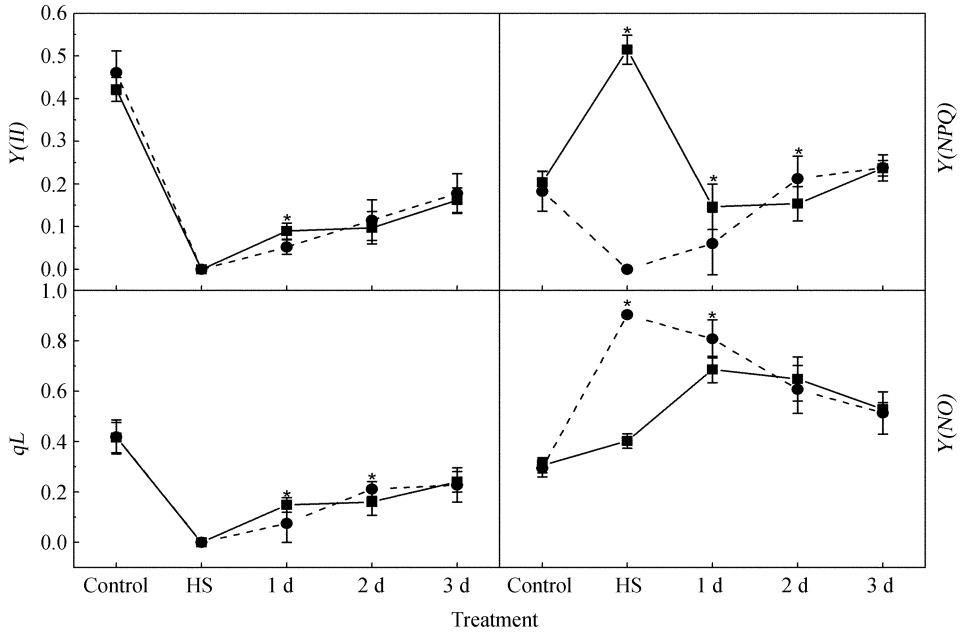

Fig. 3　The actual photochemical efficiency of PSII [$Y(II)$] and the yield of regulated energy dissipation [$Y(NPQ)$], an estimate of the fraction of open PS II centres [*qL*], and the yield of non-regulated energy dissipation [$Y(NO)$] in *A. paniculata* and *A. thaliana* upon 1 h of heat treatment at 48 ℃ with heat acclimation (37 ℃ for 2 h, 72 μmol·m^{-2}·s^{-1}) and during the subsequent recovery period. Solid and dashed lines represent *A. paniculata* and *A. thaliana*, respectively. "1 d" means 1 d of recovery at 22 ℃ after heat treatment, and so on. An asterisk means that the value in *A. paniculata* is different from that in *A. thaliana* in the same treatment ($P < 0.05$)

2.4 The induction of HSP101 affected the photochemical recovery process in *A. paniculata*

The rapid increase in HSP101 abundance after exposure to high temperature protects plants from heat damage by addressing the problems associated with protein misfolding and aggregation (Queitsch *et al.*, 2000). We compared the F_v/F_m in wild-type *A. thaliana* (Col-0) with that in an HSP101-deficient mutant (*hot-1*) after 4 h at 42 ℃ and during the subsequent recovery process at 22 ℃ to determine whether HSP101 helps to protect plays a role in the photochemical apparatus (Fig. 4A). After heat shock, F_v/F_m declined to about 0.2 in both plants. The difference of F_v/F_m between the two plants became increasingly pronounced after 4 days of recovery. This result demonstrated that HSP101 plays an important role in the photochemical repair process in *Arabidopsis*. Next, we quantified HSP101 protein in the two plants after heat treatment and during the subsequent recovery process to investigate whether and how HSP101 contributes to the process of photochemical recovery in *A. paniculata* (Fig. 4B). Although HSP101 was induced rapidly in both species after heat treatment, it was rapidly degraded after 1 d of recovery in *A. paniculata*, but maintained a high level of accumulation throughout the entire 4-day recovery process in *A. thaliana*. This indicated that the rapid induction of HSP101 in *A. paniculata* might be a signal that initiates the photochemical repair process but does not play a role in the subsequent recovery process in *A. paniculata* after moderate heat treatment. This contrasts with changes in the expression and likely role of HSP101 in *A. thaliana* after its exposure to heat stress and its subsequent recovery.

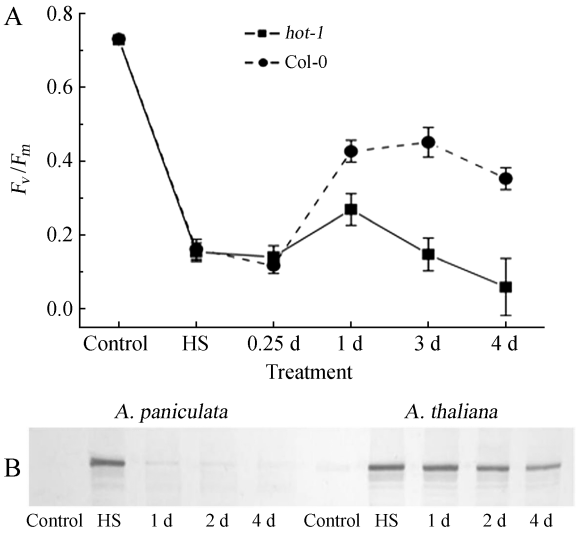

Fig. 4 Contribution of HSP101 to the photochemical recovery process in *A. paniculata* and *A. thaliana*

A. The maximum quantum yield of PSII (F_v/F_m) in *hot-1* and Col-0 of *Arabidopsis* after heat treatment (42 ℃, 4 h) and during the subsequent recovery period. Solid (with squares) and dashed lines (with circles) represent *hot-1* and Col-0, respectively. "0.25 d" means 0.25 d of recovery at 22 ℃ after heat treatment, and so on; B. The accumulation of HSP101 in *A. paniculata* and *A. thaliana* after 1 h of heat treatment at 48 ℃ with acclimation and following recovery period. "1 d" means 1 d of recovery at 22 ℃ after heat treatment, and so on

2.5 The high level of survival of *A. paniculata* seedlings under severe heat stress was consistent with its tolerance of moderate heat stress

To verify that the chlorophyll fluorescence was a reliable indicator of the heat tolerance of *A. paniculata* under a moderately high temperature, we compared the survival of this species with that of *A. thaliana* following more severe heat treatment. As shown in Fig. 5, whereas the growth of *A. paniculata* seedlings was healthy and normal, seedlings of *A. thaliana* suffered from irreversible heat damage and became etiolated after 3 h at 45 ℃ followed by 5 days of recovery at 22 ℃. This result reflected the substantial heat tolerance of *A. paniculata* under an extremely high temperature, which was consistent with its photosynthetic characterisation under moderate heat stress.

3 Conclusion

A. paniculata clearly showed strong heat tolerance under moderate stress. Compared with its relative *A. thaliana*, it presented greater photochemical efficiency after exposure to a moderately high temperature and more rapid repair of PSII. We also investigated certain physiological and biochemical factors to clarify the mechanism responsible for this rapid reversible change in photochemical efficiency of *A. paniculata* under a moderately high temperature,

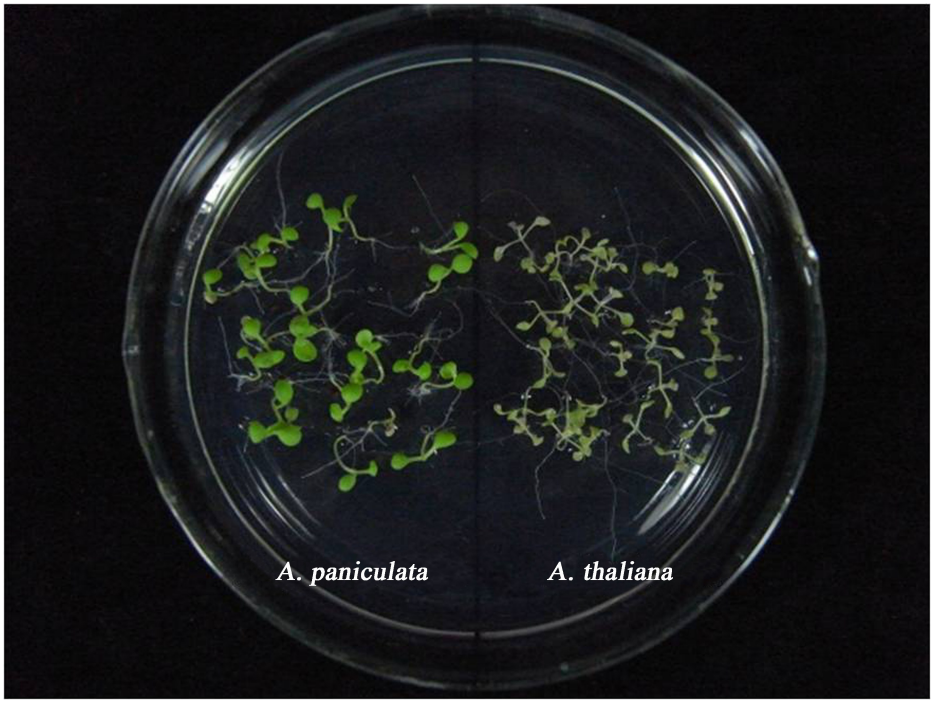

Fig. 5　Nine-day-old seedlings grown at 22 ℃ were exposed to 45 ℃ in the dark for 3 h, and were then returned to 22 ℃ for recovery. This photograph was taken after 5 days of recovery at room temperature

as experienced in the alpine environments that it inhabits. Initially, the limited increase in F_0 and more significant increase in F_m during the recovery stage were the factors that produced the rapid increase of photochemical efficiency. The dramatic and transient induction of $Y(NPQ)$ dissipated excess energy to protect the photosystem from heat damage and photodamage. Lastly but most importantly, the rapid and transient pattern of response of HSP101 after heat treatment initiated photochemical repair and ensured that this process continued smoothly. In other words, all of these traits contributed to the substantial heat tolerance of *A. paniculata*, especially in terms of its ability to continue to carry out photosynthesis under moderate stress. This was consistent with the strong thermotolerance of *A. paniculata* under extreme heat stress, which failed to cause a high rate of lethality in *A. paniculata*. Its heat tolerance system and rapid photochemical repair may constitute adaptations that enable *A. paniculata* to survive in alpine environments. Investigation of how moderate heat stress is tolerated could provide insights into how to make crops more thermotolerant through genetic modifications.

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